Validation of Plasma Irradiation Effect on Gene Transfection by Using Microplasma Jet from Capillary Nozzle

Tadashi Okihiro[1], Jun Matsuda[1], Kentaro Ikeda[1], Hideki Motomura[1], Masafumi Jinno[1], Kunihide Tachibana[2], Susumu Satoh[3], Noboru Saeki[4]

[1] *Department of Electrical and Electronic Engineering, Ehime University, 3 Bunkyo-cho Matsuyama 790-8577 Japan*
[2] *Department of Electrical and Electronic Engineering, Osaka Electro-Communication University, 18-8 Hatsucho, Neyagawa, Osaka 572-8530 Japan*
[3] *Y's Corporation, 2-3-3 Zoshigaya, Toshima, Tokyo, 171-0032 Japan*
[4] *Pearl Kogyo Co., Ltd., 3-8-13 Minami-Kagaya, Suminoe, Osaka 559-0015 Japan*
E-mail: mjin@mayu.ee.ehime-u.ac.jp

On a unique gene-transfection technique using plasma irradiation developed by some of us (Sato *et al.*), we have been studying the transfection mechanisms with various plasma sources. In this work, the effect of a microplasma jet ejected from a capillary nozzle is evaluated by irradiating plasma onto living cells in a localized area. The effect of electric field caused by the applied voltage on the sharp capillary nozzle is investigated by changing the voltage across the breakdown voltage to extract the effect of plasma irradiation.

Figure 1 shows the outline of the experimental setup. A copper capillary nozzle of 70 μm outer diameter was placed 3 mm above the sample in a Petri dish containing COS 7 cells and pCX-EGFP DNAs. The capillary nozzle worked as the high voltage electrode, while the grounded electrode was a metal wire of 160 μm diameter placed under the Petri dish. Sinusoidal voltage of 20 kHz was applied on the nozzle, which was pulse-modulated at a frequency of 25 Hz with a duty ratio of 1%. The working gas was helium with a flow rate of 100 sccm. In higher applied voltage region, filamentary plasma was generated between the capillary and the grounded wire as schematically shown in Fig. 1. The plasma was irradiated onto the sample for 4 s. In the lower applied voltage region below the breakdown voltage (see Fig. 2), the sample was only exposed to the gas flow and the high electric filed. After 24 h incubation of the treated sample, the transfection and survival rates were measured by fluorescence observation.

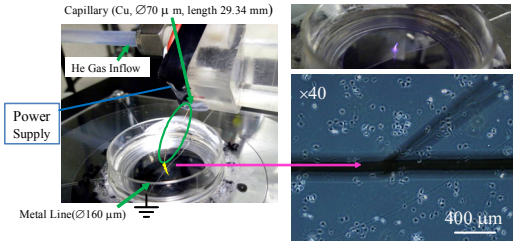

Figure 1: *Experimental setup.*

Figure 2 shows the transfection and the survival rates as a function of the applied voltage. Discharge plasmas were generated when the applied voltage was over 12 kV (peak-to-peak). The transfection occurred in this condition and the transfection rate increased exponentially with the applied voltage. These results imply that the high electric filed without plasma is not effective for the transfection. Note that the fairly high transfection rate with almost 100% cell survival is realized with this technique.

This work was partly supported by the Grant-in-Aid (22654070) from JSPS.

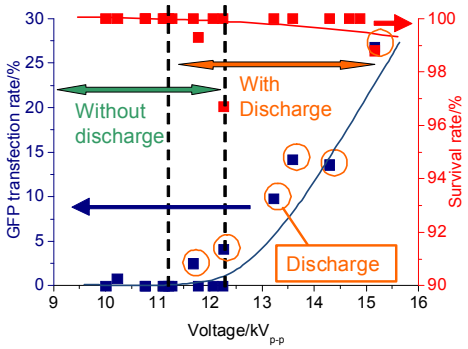

Figure 2: *The transfection and the survival rate vs. applied voltage.*